Baseline and Stress-Induced Plasma Corticosterone during Long-Distance Migration in the Bar-Tailed Godwit, Limosa lapponica

Landys-Ciannelli, M.M.; Ramenofsky, M.; Piersma, T.; Jukema, J.; Castricum Ringing Group; Wingfield, J.C.

Published in:
Physiological and Biochemical Zoology

DOI:
10.1086/338285

Baseline and Stress-Induced Plasma Corticosterone during Long-Distance Migration in the Bar-Tailed Godwit, *Limosa lapponica*

Mėta M. Landys-Ciannelli[1,*]
Marilyn Ramenofsky[1]
Theunis Piersma[2,3]
Joop Jukema[4]
Castricum Ringing Group[5]
John C. Wingfield[1]
[1]Department of Zoology, Box 351800, University of Washington, Seattle, Washington 98195; [2]Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands; [3]Centre for Ecological and Evolutionary Studies, Zoological Laboratory, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands; [4]Haerdawei 44, 8854 AC Oosterbierum, Friesland, The Netherlands; [5]c/o Arnold Wijker, Bergstraat 39, 1931 EN Egmond aan Zee, The Netherlands

Accepted 10/23/01

ABSTRACT

The specific roles of corticosterone in promotion of avian migration remain unclear even though this glucocorticosteroid is elevated in many migrating bird species. In general, glucocorticosteroids promote metabolic homeostasis and may elicit effects on feeding and locomotion. Because the migratory stages of refueling and flight are characterized by distinct behaviors and physiology, the determination of corticosterone levels during each stage should help identify potential processes in which corticosterone is involved. We measured baseline levels of corticosterone in bar-tailed godwits (*Limosa lapponica*) during two distinct stages of migration: (1) immediately after arrival at a false stopover site just short of the Wadden Sea and (2) throughout the subsequent 4-wk refueling period on the Wadden Sea. Plasma corticosterone was higher in arriving than in refueling birds. In addition, corticosterone increased with size-corrected body mass during the refueling phase, suggesting that corticosterone rises as birds prepare to reinitiate flight. Therefore, elevated corticosterone appears associated with migratory flight and may participate in processes characterizing this stage. We also performed a capture stress protocol in all birds and found that corticosterone increased in both arriving and refueling godwits. Therefore, the normal course of migration may be typified by corticosterone concentrations that are lower than those associated with stressful and life-threatening episodes.

* Corresponding author; e-mail: meta@u.washington.edu.

Physiological and Biochemical Zoology 75(1):101–110. 2002. 1522-2152/2002/7501-00117$15.00

Introduction

Many species of birds migrate thousands of kilometers between summer breeding grounds and winter feeding sites. Most birds complete each migratory journey in multiple bouts of flight to allow for intermittent refueling in stopover areas. Thus, they alternate between physiological stages of flight and fueling. In addition to obvious differences in behavior, the physiology of birds in these stages is unique; migratory flight is characterized by prolonged bouts of energy expenditure at up to seven times basal metabolic rate (Alerstam 1990; Butler and Woakes 1990), while refueling is characterized by unusually rapid energy deposition (Ramenofsky 1990; Jenni-Eiermann and Jenni 1991).

Hormones are likely involved in the promotion of these migratory behaviors and associated physiological processes because they allow for quick redirection and maintenance of behavior and physiology. Reproductive steroids, thyroid hormones, insulin, prolactin, and corticosterone have been implicated in migration, although their roles remain to be resolved (reviewed in Wingfield et al. 1990). To date, evidence for the involvement of corticosterone in migration is most clear. First, the activity of adrenocortical tissue (the site of corticosterone synthesis) increases in many migrating birds (Naik and George 1963; Lorenzen and Farner 1964; John 1965; Peczely 1976). Second, elevated plasma levels of corticosterone have been identified during the course of migration in many species (O'Reilly 1995; Holberton et al. 1996; Romero et al. 1997; Piersma and Ramenofsky 1998; Holberton 1999; Piersma et al. 2000).

In general, glucocorticosteroids such as corticosterone stimulate de novo glucose synthesis (Hadley 1996) and elicit effects on feeding (King 1987; Tempel and Leibowitz 1989; Dallman et al. 1993) and locomotion (Veldhuis et al. 1982; Astheimer et al. 1992; Bruener et al. 1998). Although research suggests that levels of corticosterone are elevated during migration, to our knowledge, only two studies have examined a possible role

of elevated corticosterone in relation to migration. Dolnik and Blyumental (1967) found that injections of cortisol increased daytime activity of premigratory chaffinches (*Fringilla coelebs*; a daytime migrant) to levels seen in migrating conspecifics. Meier and Martin (1971) found that injections of corticosterone increased daytime and nocturnal activity in photosensitive white-throated sparrows (*Zonotrichia albicollis*; a nighttime migrant) placed on long days. These studies suggest that corticosterone may induce *Zugunruhe*, or migratory restlessness.

The determination of plasma corticosterone concentrations at different stages of migration might help elucidate the migratory role(s) of corticosterone. Because each stage of migration is characterized by distinct behavior and physiology, differences in corticosterone concentration between stages could suggest the correlated processes possibly affected by corticosterone. To date, few attempts have been made to document corticosterone patterns during migration because migratory stages in the field are difficult to assess (Wingfield et al. 1990).

Bar-tailed godwits (*Limosa lapponica*) are ideal for investigating the relationship between plasma corticosterone and migratory stage. Godwits depart synchronously (Piersma et al. 1990) from wintering sites in West Africa and arrive on breeding areas in northern Russia after making only one midway stopover in the Wadden Sea of the Netherlands, Germany, and Denmark (Cramp and Simmons 1983; Drent and Piersma 1990). Therefore, the migratory stage of an individual godwit can be readily identified.

Preliminary research by Ramenofsky et al. (1996) suggests that in response to capture, godwits that recently arrived at the Wadden Sea have a higher potential to secrete corticosterone than do birds that are refueling. We therefore hypothesize that plasma corticosterone is not uniformly elevated during migration but rather fluctuates in concentration according to migratory stage (i.e., according to the expression of specific behaviors and physiological processes).

To identify the pattern of plasma corticosterone during migration, we measured baseline levels of corticosterone in godwits during two distinct migratory stages: (1) immediately after their arrival at a false stopover site just short of the Wadden Sea and (2) throughout their subsequent 4-wk refueling period on the Wadden Sea stopover area. Godwits were also subjected to a standardized capture stress protocol to simulate an unpredictable, and therefore stressful, event within the life-history stage of migration (Wingfield et al. 1992; Wingfield and Ramenofsky 1999), whereby we examined the sensitivity of the corticosterone secretory axis to stressors in the environment (Wingfield et al. 1998; Wingfield and Ramenofsky 1999), the capacity of migrating godwits to secrete corticosterone, and the relationship between energetic condition and stress-induced corticosterone levels.

Material and Methods

Study Animals

The bar-tailed godwits sampled in this study winter in Mauritania and Guinea-Bissau, West Africa. They breed on the Taimyr Peninsula, Russia (Boere and Smit 1981; Cramp and Simmons 1983; Piersma and Jukema 1990) and have recently been assigned the subspecies status *Limosa lapponica taymyrensis* (Engelmoer and Roselaar 1998). During spring migration, bar-tailed godwits travel the 9,000-km distance between their wintering sites and breeding grounds in two bouts of approximately equal length. Godwits begin to fatten in March (Piersma 1987) and depart from West Africa in late April (Piersma et al. 1990). They then fly 4,500 km nonstop to their main refueling site in the Wadden Sea (Prokosch 1988; Piersma and Jukema 1990). Bar-tailed godwits spend about 4 wk staging in the Wadden Sea area before initiating the 4,500-km flight to their northern breeding grounds (Boere and Smit 1981; Prokosch 1988; Piersma and Jukema 1990; M. Green, T. Piersma, J. Jukema, and P. de Goeij, unpublished manuscript).

Godwits normally fly from West Africa to the Wadden Sea without stopping to refuel. The average date of departure from West Africa and average date of arrival at the Wadden Sea correspond closely to predicted flight time, assuming no stopovers en route (Fig. 1). In fact, feces of arriving godwits are green with bile, as would be expected of birds that have not been feeding (feces of red knots *Calidris canutus* that have been food deprived for several days are green; T. Piersma, personal observation). Therefore, we are confident that birds arriving in the Wadden Sea have just completed a flight bout of about 4,500 km.

We captured migrating bar-tailed godwits at two sites: (1) in the dunes near Castricum (52°32′N, 04°37′E), located on the western coast of the Netherlands and just short of the Wadden Sea, and (2) on Texel (53°03′N, 04°48′E), a Dutch island in the Wadden Sea. Because godwits usually migrate only after reaching their second year (Cramp and Simmons 1983), birds studied here are adults.

Godwits captured in the dunes of Castricum (a site directly on the flight route from West Africa; Piersma 1987; Fig. 1) were intercepted during flight only 60 km short of the Wadden Sea (see Landys et al. 2000). Godwits do not normally land in Castricum during spring migration but rather regularly fly overhead from late April to mid-May. However, overflying birds can be lured into landing during daylight hours with song playbacks and decoys. The dunes of Castricum are used for the purification of water for human consumption and so contain many man-made bodies of water. Luring birds down in Castricum may be possible only because birds are already close to their normal stopover destination and so anticipate an imminent refueling stop and because the man-made water systems around the capture area simulate such a potential refueling site. In addition, capture may be facilitated by the low altitudes at

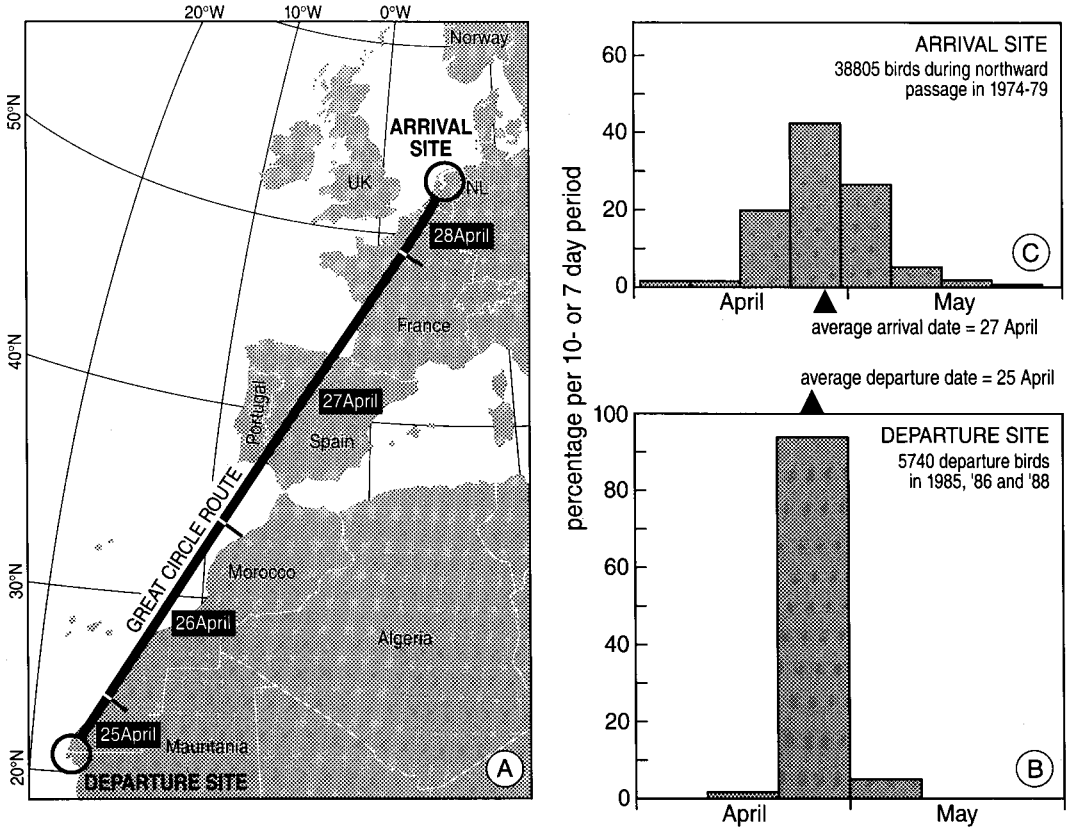

Figure 1. *A*, Approximate flight route for migrating bar-tailed godwits after departure from West Africa in spring (Piersma 1987). The flight seems to follow a great circle route. The predicted flight time from West Africa is 2.6 d (Piersma 1987). *B*, Timing of spring departure of godwits from Banc d'Arguin, Mauritania. Departure observations are from Piersma et al. (1990). Departure is synchronized, with the majority of birds leaving within a period of about 10 d. The average date of departure is April 25. *C*, The timing of the arrival of godwits to the coast of North Holland, near the Castricum capture site. Observations are from Camphuysen and van Dijk (1983). Average date of arrival is April 27, suggesting an average flight time from West Africa to North Holland of <3 d, as predicted by Piersma (1987). Over 90% of the arriving birds in North Holland were sighted before May 6. The lack of departure sightings from the Banc d'Arguin in early April suggests that the godwits arriving in the Netherlands at this time may not originate from Banc d'Arguin.

which birds overfly Castricum (Piersma and van de Sant 1992) because they are then better able to hear and react to song playbacks.

At Castricum, arriving godwits were captured with clap nets immediately upon landing from April 27 to May 7, 1997–1999. We are confident that these godwits are not a biased sample of unnaturally exhausted or inexperienced birds. First, body mass (minus estimated intestinal contents: 3.3 and 8.5 g for arriving and refueling males, respectively) of male godwits captured in Castricum is equivalent to that of male godwits refueling at an early touchdown site (not Texel) in the Wadden Sea (average ± SE: 190.8 ± 1.8 and 198.5 ± 5.3 g, respectively; Student's *t*-test: $t = -1.520$, $P = 0.133$). Estimated intestinal content mass was based on body composition analyses of a different subset of birds (T. Piersma, personal observation). We were unable to compare the mass of arriving and early refueling females due to low sample size. Second, godwits that are lured to land in Castricum do so in flocks and not as single individuals, suggesting that captured birds are not atypical "drop-outs." Therefore, we consider that godwits captured in Castricum are examples of normal migrants near the end of a long bout of flight and henceforth refer to them as "arriving" godwits.

On the Wadden Sea island of Texel, godwits were captured during daylight hours with a large pull net, the "wilsternet" (Koopman and Hulscher 1979; Jukema et al. 2001), in the latter three-quarters of the stopover period (i.e., from May 11 to June 1, 1996–1999). In 6 yr of observations, 91.4% of godwits arrived into the Wadden Sea area before May 6 (Camphuysen and van Dijk 1983; Fig. 1). At the earliest, we began to catch birds on Texel on May 11 (godwits do not initiate refueling in the Texel grasslands until the first fields have been mown in mid-May).

Therefore, the majority of godwits captured on Texel were refueling birds and not late arrivals. We henceforth refer to godwits captured on Texel as "refueling" birds.

Sampling

Baseline levels of corticosterone were determined from blood samples collected within 4 min after birds were netted. A subset of birds caught in 1996, 1997, and 1998 were each again sampled at 10, 30, and 60 min after capture to examine corticosterone levels attained in response to capture and handling. This capture-and-handling stress protocol (Wingfield et al. 1992) is a reproducible technique that simulates stress within a normal life-history stage of an organism, "stress" being defined as a response to an unpredictable and noxious stimulus in the environment (Wingfield et al. 1998; Wingfield and Ramenofsky 1999). Birds were held in large cloth bags or wooden crates between samplings. Godwits were banded, weighed, measured, and sexed as described in Piersma and Jukema (1990) and then released.

Blood samples were obtained by puncturing the brachial vein with a sterile 23-gauge needle and collecting the blood into heparinized microhematocrit capillary tubes. Samples were immediately stored on ice and centrifuged within 10 h of collection at 6,900 *g* for 10 min. Aspirated plasma was placed into microcentrifuge tubes, stored at −80°C, transported to the University of Washington, Seattle, on dry ice, and subsequently stored at −20°C. The corticosterone concentration in each sample was determined by direct radioimmunoassay, following the procedure described in Wingfield et al. (1992) and used previously for this population of godwits (Ramenofsky et al. 1996). Plasma samples of 20 μL were combined with 180 μL distilled H_2O. For the determination of percentage recovery of steroid, 2,000 cpm ^{3}H-corticosterone (NEN : NET-399) was added and allowed to equilibrate for at least 30 min at 4°C. The organic phase was extracted for 2 h with 4 mL redistilled dichloromethane and dried under nitrogen gas at 37°C. Dried extracts were resuspended in 550 μL phosphate-buffered saline with 0.1% gelatin. Duplicate samples of 200 μL were used for the radioimmunoassay (RIA). Corticosterone concentration was calculated from a standard curve that ranged from 7.8 to 2,000 pg/mL. Of the remaining resuspended extract, 100 μL was used to determine the percent steroid recovered after extraction. Minimum average recoveries between assays were 77%. Corticosterone concentrations determined from RIA were adjusted to account for percentage steroid lost during extraction. A total of 11 assays were run. Intra-assay variation, as calculated from duplicate samples, was 4%. Interassay variation, as calculated from a known standard, was 14%.

Data Analysis

The bar-tailed godwit is sexually dimorphic, with females larger than males (Piersma and Jukema 1990). Previous studies have often tried to correct for sex-related size differences by dividing body mass by a measure of body size (Piersma et al. 1996) or have simply performed analyses on males and females separately (Piersma 1998). The latter method dramatically decreases sample size and, thus, statistical power. The use of ratios is also unsatisfactory unless a trait varies isometrically with body size (Packard and Boardman 1987, 1988). A superior method for the construction of a size-corrected body mass measure is the determination of mass residuals from a regression against body size (Bennett 1987; Packard and Boardman 1987, 1988).

We determined mass residuals for all godwits to correct for sex-related mass differences. Sex and body-size data from refueling godwits were introduced into a multiple linear regression model. This analysis revealed that log wing length alone was significant in predicting log body mass and that the slopes of the female and male regression lines were equal. We calculated mass residuals for refueling and arriving birds from the regression of log body mass against log wing length (according to the slope 2.758) for both sexes combined. Mass residuals are called "size-corrected body mass" in the "Discussion" section and are used as an indirect index of energetic condition. We back transformed all mass residuals to a normalized female body mass estimate (in grams) for use in figures by adding mass residuals to the average of log female body mass and taking the antilog.

We collected data for both arriving and refueling godwits during the years 1997, 1998, and 1999. In contrast, in 1996, we were able to sample only refueling godwits. Therefore, when comparing arriving and refueling birds, we consider data only from the years 1997 to 1999. When looking at patterns within the stage of refueling, we also consider data from 1996.

When using ANCOVA tests (which fit parallel slopes through data groups and then test for differences in elevation between lines), we verified that slopes were homogeneous, as required by the use of the ANCOVA. Unless otherwise noted, Tukey tests were used for the examination of pairwise differences in all post hoc tests. Parametric tests met normality and constant variance requirements except in one noted case. If data were used for more than one test, then the critical probability α was Bonferroni adjusted according to the number of tests used (Sokal and Rohlf 1995). The statistical packages SYSTAT 10 and SigmaStat 2.0 were used for all analyses (SPSS 1997; Wilkinson 2000).

Differences in mass residuals between the migratory stages of arrival and stopover were tested with a Mann-Whitney rank sum test. To test for annual differences in mass gain during stopover, we entered mass residuals from refueling birds into an ANCOVA model, in which we included year as a factor and day of sampling as a covariate.

We $\log_{10}$ transformed baseline corticosterone values to normalize data for use in statistical analyses. Preliminary tests indicated that baseline corticosterone did not vary with time of day or sex. In addition, effects of active body molt on baseline levels of corticosterone could be explained by correlated mass differences (see Piersma and Jukema 1993). We therefore excluded the variables time of day, sex, and molt from subsequent models.

We used a Student's *t*-test to compare baseline levels of corticosterone between arriving and refueling birds. We then tested for patterns in baseline corticosterone within the stopover period. An ANCOVA was used to examine how mass residuals of refueling birds affect baseline corticosterone. Year was included as a factor and mass residuals were included as a covariate. Entered data did not satisfy the constant-variance test (Sokal and Rohlf 1995; Zar 1996).

Corticosterone data collected during the capture-and-handling stress protocol were not normally distributed and could not be transformed to satisfy normality requirements. Therefore, we were unable to directly compare the stress response between arriving and refueling godwits with a two-way repeated-measures test. Rather, we examined the effects of capture and handling on plasma corticosterone in arriving and in refueling birds separately. The effect of capture stress on arriving birds was examined with a one-way repeated-measures ANOVA. In refueling birds, this effect was examined with a nonparametric Friedman repeated-measures ANOVA on ranks. A pairwise Dunn's post hoc test was used to examine differences in corticosterone level among the sampling times in refueling birds. Untransformed serial samples collected at baseline, 10, 30, and 60 min were included in the repeated-measures tests.

We determined the maximum level of corticosterone achieved by each bird during the capture-and-handling stress protocol by selecting the highest plasma concentration of corticosterone among the four samples collected for each individual. The timing of this peak corticosterone level (baseline, 10, 30, or 60) was recorded. Maximum levels of corticosterone between arriving and refueling birds were compared with an ANCOVA. Migratory stage was included in the model as a factor and mass residuals were included as a covariate. Corticosterone data were log transformed to meet normality requirements. The timing of the corticosterone peak was compared between arriving and refueling birds with a nonparametric Mann-Whitney rank sum test.

Results

Body mass residuals were significantly lower at arrival than during refueling (Mann-Whitney rank sum test: $U = 95.000$, $P < 0.001$). Arriving males and females weighed 194.0 ± 2.5 and 237.4 ± 3.8 g, respectively, suggesting that they had lost 55.4% and 55.2% of their body mass during the 4,500-km flight from Banc d'Arguin, Mauritania, to the Wadden Sea (departure masses were taken from Piersma and Jukema [1990]). This estimated mass loss is higher than predicted by Piersma and Jukema (1990). However, preliminary data on body composition indicate that arriving birds still carry residual fat and thus do not represent a compromised or starved condition (T. Piersma, personal observation).

In refueling birds, mass residuals increased significantly with sampling day (ANCOVA: $F = 49.712$, df = 1,235, $P < 0.001$; Fig. 2) and also varied with year (ANCOVA: $F = 13.647$, df = 3,235, $P < 0.001$). Mass residuals were significantly lower in 1999 than in other years (Tukey: $P < 0.001$; Fig. 2).

Baseline levels of corticosterone were higher at arrival than during refueling (18.0 ± 1.6 and 8.8 ± 0.7 ng mL^{-1}, respectively; Student's *t*-test: $t = 6.173$, $P < 0.001$; Fig. 3). In refueling birds, baseline corticosterone varied positively with mass residuals (ANCOVA: $F = 6.634$, df = 1,235, $P = 0.011$; Fig. 3), and also according to year (ANCOVA: $F = 15.503$, df = 3,235, $P < 0.001$). Baseline levels in refueling birds were similar in 1998 and 1999 (Tukey: $P > 0.05$). Baseline levels were higher in 1996 than in 1997 and were lowest in 1998 and 1999 (Tukey: $P < 0.05$).

During the stress of capture and handling, plasma corticosterone increased significantly in both arriving (repeated-measures ANOVA: $F = 10.231$, df = 3,63, $P < 0.001$) and in refueling birds (Friedman repeated-measures ANOVA on ranks: $X_3 = 138.914$, $P < 0.001$; Fig. 4). In arriving birds, corticosterone levels at baseline were lower than corticosterone levels at 10, 30, and 60 min after capture (Tukey: $P < 0.05$) and corti-

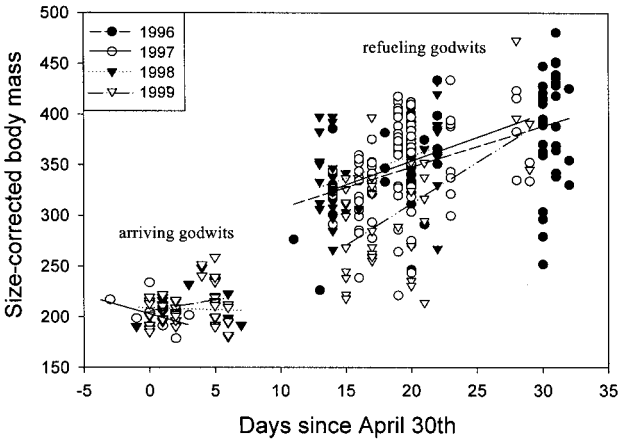

Figure 2. Size-corrected body mass of bar-tailed godwits arriving at and refueling on the Wadden Sea stopover site. Arriving birds were caught April 27–May 7, and refueling birds were caught May 11–June 1. The mass of refueling godwits was significantly higher than that of arriving birds. During stopover, body mass significantly increased with sampling day and was also different between years. Specifically, body mass was lowest in 1999. Data from both stages and all 4 yr are shown. Arriving birds were not sampled in 1996.

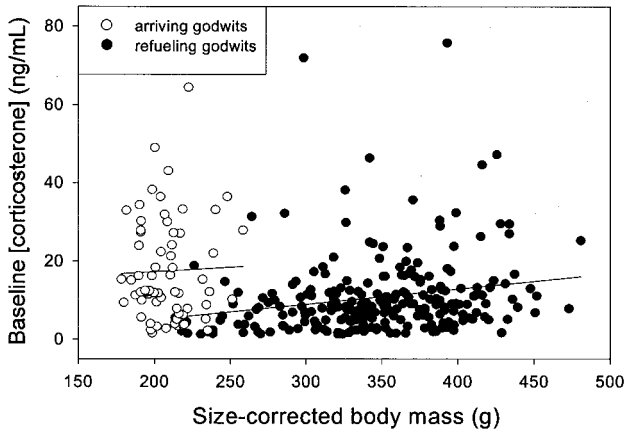

Figure 3. Baseline plasma corticosterone of bar-tailed godwits arriving at and refueling on the Wadden Sea stopover site. Baseline levels are regressed against size-corrected body mass. Corticosterone was significantly higher in arriving godwits than in refueling birds. In refueling birds, corticosterone correlated positively with size-corrected body mass. Baseline corticosterone values from both stages and all 4 yr are shown. Even though the slopes between arriving and refueling birds were not compared, a slope is drawn through data from arriving birds for purposes of presentation.

costerone levels in samples collected at 10, 30, and 60 min after capture were not significantly different from each other (Tukey: $P > 0.05$). In refueling birds, pairwise comparisons showed that except for samples collected at 30 and 60 min after capture (Tukey: $P > 0.05$), corticosterone levels at each of the different sampling points were significantly different from each other (Tukey: $P < 0.05$).

Maximum levels of corticosterone did not differ between arriving and refueling birds (64.2 ± 6.9 and 66.4 ± 2.6 ng mL^{-1}, respectively; ANCOVA: $F = 0.173$, df $= 1,78$, $P = 0.678$; Fig. 4) and were independent of mass residuals (ANCOVA: $F = 0.006$, df $= 1,78$, $P = 0.941$; Fig. 5). However, maximal levels of corticosterone occurred significantly earlier during the capture stress protocol in arriving godwits than in refueling birds (Mann-Whitney rank sum test: $U = 336.5$, $P = 0.014$).

Discussion

Plasma Corticosterone Patterns in Migrating Bar-Tailed Godwits

Capitalizing on the synchronous migration of bar-tailed godwits (Piersma et al. 1990), our ability to lure them down at the end of a long-distance flight, and the accessibility of their stopover site, we have documented levels of baseline and of stress-induced plasma corticosterone during the migratory stages of arrival and refueling. Earlier studies identified a general pattern of increased adrenal activity and elevated plasma corticosterone in various migrating bird species (Naik and George 1963; Lorenzen and Farner 1964; John 1965; Peczely 1976; O'Reilly 1995; Holberton et al. 1996; Romero et al. 1997; Piersma and Ramenofsky 1998; Holberton 1999; Piersma et al. 2000) but did not attempt to identify how plasma levels of corticosterone change during the migratory season. We found that baseline levels of corticosterone are related to migratory stage. First, baseline levels of corticosterone were higher in godwits arriving at their stopover site than in refueling birds. Second, baseline corticosterone positively correlated with size-corrected body mass on the stopover site. Assuming that heavier birds are the first to depart the stopover site, the latter corticosterone pattern suggests that plasma levels rise in fueled birds preparing to reinitiate migratory flight. However, no mass threshold was apparent (cf. Fig. 3), indicating that additional factors may influence the timing of corticosterone secretion or of departure.

Fueling was delayed in 1999 (Fig. 2), possibly as a consequence of the relatively lower density of leatherjackets (Tipulidae larvae; J. Jukema, personal observation), an important prey item on the stopover site (Piersma et al. 1993). In conjunction with the delay in refueling, corticosterone levels on the stopover site were low. Therefore, 1999 stopover data support the idea that higher corticosterone levels correlate with completion of fueling and departure.

Different sampling schedules between years may have contributed to the interannual variability of plasma corticosterone. We were unable to capture birds toward the end of the stopover period in 1998 (cf. Fig. 2). The correlated lower corticosterone

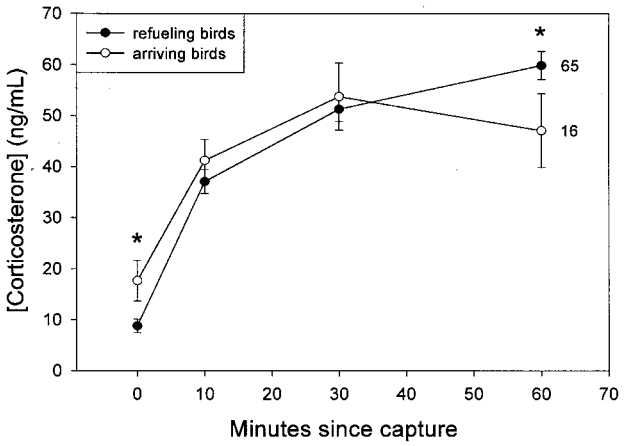

Figure 4. The response of plasma corticosterone to capture-and-handling stress in arriving and in refueling godwits. Baseline samples were taken within 4 min of capture. Samples were also collected at 10, 30, and 60 min after capture. Corticosterone increased with time after capture both in arriving and in refueling birds. In arriving birds, corticosterone levels stopped increasing after the 10-min sampling point, whereas in refueling birds, levels stopped increasing only after the 30-min sampling point. The corticosterone values shown are untransformed. Sample sizes are displayed. Error bars indicate SEs.

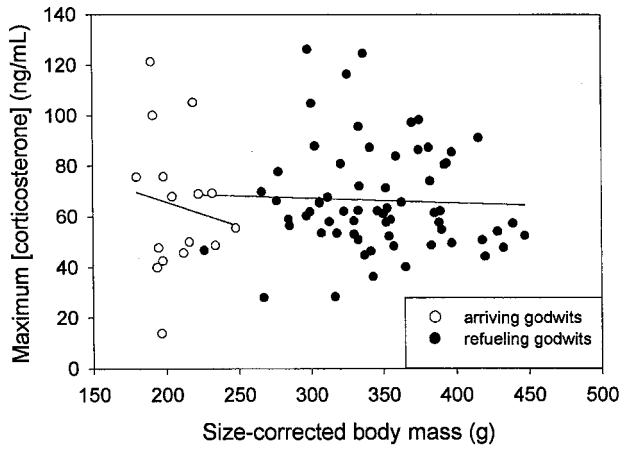

Figure 5. Maximum levels of corticosterone in relation to size-corrected body mass of both arriving and refueling godwits. Energetic condition did not determine the capacity to secrete corticosterone. Data shown are untransformed.

levels in this year may be more representative of refueling birds and not of birds preparing to depart the stopover site. Clearly, more studies are needed to explain interannual differences in corticosterone during refueling.

Plasma corticosterone increased more than threefold both in arriving and in refueling godwits that were subjected to the capture-and-handling stress protocol (cf. Fig. 4). Therefore, baseline levels of corticosterone even at the end of a migratory flight reside far below those associated with acute and unpredictable stressors such as capture. Previous research on various migrant species confirms that plasma corticosterone generally resides below maximal levels during migration (Schwabl et al. 1991; Gwinner et al. 1992; O'Reilly 1995; Romero et al. 1997; Tsipoura et al. 1999; Mizrahi et al. 2001). Thus, in spite of the high energetic demands encountered during flight and the exertion required to complete travel, relatively low baseline levels of corticosterone in arriving and in refueling godwits suggest that migration in itself does not constitute a stress but, rather, that it is a predictable and normal stage within the life-history cycle of a migratory individual (Wingfield et al. 1998).

Maximum levels of corticosterone were not different between arriving and refueling godwits, suggesting that birds in different stages of migration have a similar capacity to secrete corticosterone. However, maximum levels of corticosterone occurred significantly earlier during the capture stress protocol in arriving than in refueling birds. This result is supported by post hoc analyses of the stress series data; plasma corticosterone reached a plateau at 10 min after the start of sampling in arriving birds, whereas the corticosterone of refueling birds continued to increase until 30 min after the start of sampling (cf. Fig. 4), indicating that within the sampling schedule, refueling birds reach maximum corticosterone levels later than arriving birds. We therefore suggest an increased sensitivity of the corticosterone secretory axis in arriving as compared to refueling godwits (Hadley 1996). Up-regulated sensitivity of the axis may allow flying migrants to more quickly adjust behavior and physiology to maintain homeostasis during long-distance flight (Holberton et al. 1998; Holberton and Able 2000). On the other hand, the quicker rise in corticosterone levels in arriving birds may be a consequence of their already elevated baseline levels.

Individuals with low energy reserves are more likely to be affected by adverse conditions and typically exhibit higher plasma levels of corticosterone in response to stress (Cherel et al. 1988; Wingfield et al. 1994; Hood et al. 1998; Kitaysky et al. 1999*a*, 1999*b*). However, maximum levels of corticosterone in migrating godwits did not change in relation to size-corrected body mass (Fig. 5). The lack of a relationship between capacity to secrete corticosterone and energetic condition may suggest that successfully migrating godwits are far from a compromised or starved condition.

Possible Functions of Corticosterone

In our study, baseline levels of corticosterone peaked at the conclusion of a 4,500-km bout of migratory flight and during preparations for the subsequent 4,500-km bout. Given the obvious difficulty of sampling flying birds, we were unable to determine corticosterone levels of godwits in midflight during either of these two bouts. However, when taken to represent one complete bout, collected data suggest that baseline levels of plasma corticosterone are low during the start of refueling but rise in preparation for, and remain elevated during, flight. Corticosterone may therefore differentially participate in processes during the fueling and flight stages.

We suggest that elevated baseline levels of corticosterone during migration may primarily aid in energy mobilization toward the end of refueling and during flight (Nazir et al. 1988; Santana et al. 1995; Hadley 1996). This point is discussed at greater length below. Elevated corticosterone may also stimulate migratory restlessness (Dolnik and Blyumental 1967; Meier and Martin 1971) and increase general awareness to permit successful completion of travel in unpredictable conditions (Piersma et al. 2000); even though migration occurs predictably each year, local weather conditions and prey availability are often variable (Holberton et al. 1998).

Daily fluctuations in glucocorticosteroids correlate with onset of feeding and activity in many species (e.g., Joseph and Meier 1973; Lauber et al. 1987; Dallman et al. 1993; Breuner et al. 1999). Dallman et al. (1993) suggest that peaks in glucocorticosteroids anticipate ingestion-associated metabolic demands. Although godwits do not feed during flight, elevated levels of corticosterone in godwits preparing for departure may similarly act to up-regulate metabolic functions for anticipated future energetic needs. Therefore, an elevated baseline in de-

parting godwits may signify entry into a heightened metabolic state. Because corticosterone baseline is also high in arriving godwits, we suggest that corticosterone continues to promote metabolic processes, such as the stimulation of de novo glucose synthesis and the mobilization of lipid stores during the energetically demanding phase of flight (Nazir et al. 1988; Santana et al. 1995; Hadley 1996). Indeed, plasma-free fatty acids and glycerol levels were significantly higher in arriving birds than in birds refueling on the stopover site (M. Landys, personal observation), indicating increased lipid mobilization in conjunction with elevated levels of corticosterone.

In summary, we suggest that corticosterone levels during migration correlate with the stage of flight, to possibly promote heightened metabolic processes. Elevated levels of corticosterone in pigeons that have recently completed a bout of flight (Haase et al. 1986) support our proposed model. In addition, our data indicate that plasma corticosterone rises in anticipation of migratory flight. During the migratory period, captive yellow-rumped warblers (*Dendroica coronata*) and captive red knots also increase corticosterone titers after attaining a body mass typical of departure, even though they do not actually initiate migratory flight (Holberton 1999 and Piersma et al. 2000, respectively). Therefore, corticosterone not only may increase as a consequence of flight-associated metabolic demands but may play an additional regulatory role during migration.

Obviously, corticosterone is not the only peripheral signal involved in migratory behavior and physiology. Once the role of corticosterone has been clarified, studies can begin to examine how other hormones and their interactions with corticosterone promote the complex process of migration.

Acknowledgments

We thank Petra de Goeij, Pieter Honkoop, Silke Nebel, Jeroen Reneerkens, and Yvonne Verkuil for help in the field; Lynn Erckmann and Ciska Raaijmakers for assistance in the laboratory; Dick Visser for drafting the first figure; Ray Huey, Sarunas Landys, Ignacio Moore, Jeroen Reneerkens, and four anonymous reviewers for valuable suggestions on earlier drafts of this manuscript; and Lorenzo Ciannelli, Wolfgang Goymann, and Alexander Kitaysky for help in the field of statistics. We are grateful to the Naamloze Vennootschap Provinciaal Waterleidingbedrijf van Noord-Holland for providing lodging to M.M.L.-C. in the dune reserve. Project work was supported by a fellowship awarded to M.M.L.-C. by the Seattle chapter of the Achievement Rewards for College Scientists Foundation, by a PIONIER grant awarded to T.P. from the Netherlands Organization for Scientific Research, and by a National Science Foundation grant from the Office of Polar Programs awarded to J.C.W. This is publication number 3,499 of the Netherlands Institute of Sea Research.

Literature Cited

Alerstam T. 1990. Bird Migration. Cambridge University Press, Cambridge.

Astheimer L.B., W.A. Buttemer, and J.C. Wingfield. 1992. Interactions of corticosterone with feeding, activity and metabolism in passerine birds. Ornis Scand 23:355–365.

Bennett A.F. 1987. Interindividual variability: an underutilized resource. Pp. 147–169 in M.E. Feder, A.F. Bennett, W.W. Burggren, and R.B. Huey, eds. New Directions in Ecological Physiology. Cambridge University Press, Cambridge.

Boere G.C. and C.J. Smit. 1981. Bar-tailed godwit (*Limosa lapponica*). Pp. 170–179 in C.J. Smit and W.J. Wolff, eds. Birds of the Wadden Sea. Balkema, Rotterdam.

Breuner C.W., A.L. Greenberg, and J.C. Wingfield. 1998. Noninvasive corticosterone treatment rapidly increases activity in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Gen Comp Endocrinol 111:386–394.

Breuner C.W., J.C. Wingfield, and L.M. Romero. 1999. Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. J Exp Zool 284:334–342.

Butler P.J. and A.J. Woakes. 1990. The physiology of bird flight. Pp. 300–318 in E. Gwinner, ed. Bird Migration: Physiology and Ecophysiology. Springer, Berlin.

Camphuysen C.J. and J. van Dijk. 1983. Seabirds and estuary birds along The Netherlands' coast, 1974–79. Limosa 56: 87–230.

Cherel Y., J.P. Robin, O. Walch, H. Karmann, P. Netchitailo, and Y. Le Maho. 1988. Fasting in the king penguin. I. Hormonal and metabolic changes during breeding. Am J Physiol 23:R170–R177.

Cramp S. and K.E.L. Simmons. 1983. Handbook of the Birds of Europe, North Africa and the Middle East—the Birds of the Western Palaearctic. Vol. 3. Waders to Gulls. Oxford University Press, Oxford.

Dallman M.F., A.M. Strack, S.F. Akana, M.J. Bradbury, E.S. Hanson, K.A. Scribner, and M. Smith. 1993. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Front Neuroendocrinol 14:303–347.

Dolnik V.R. and T.I. Blyumental. 1967. Autumnal premigratory and migratory periods in the chaffinch (*Fringilla coelebs coelebs*) and some other temperate zone passerine birds. Condor 69:435–468.

Drent R. and T. Piersma. 1990. An exploration of the energetics of leap-frog migration in arctic breeding waders. Pp. 399–412 in E. Gwinner, ed. Bird Migration: Physiology and Ecophysiology. Springer, Berlin.

Engelmoer M. and C.S. Roselaar. 1998. Geographical Variation in Waders. Kluwer, Dordrecht.

Gwinner E., M. Zeman, I. Schwabl-Benzinger, S. Jenni-Eiermann, L. Jenni, and H. Schwabl. 1992. Corticosterone levels of pas-

serine birds during migratory flight. Naturwissenschaften 79: 276–278.

Haase E., A. Rees, and S. Harvey. 1986. Flight stimulates adrenocortical activity in pigeons (*Columba livia*). Gen Comp Endocrinol 61:424–427.

Hadley M.E. 1996. Endocrinology. 5th ed. Prentice Hall, Upper Saddle River, N.J.

Holberton R.L. 1999. Changes in patterns of corticosterone secretion concurrent with migratory fattening in a Neotropical migratory bird. Gen Comp Endocrinol 116:49–58.

Holberton R.L. and K.P. Able. 2000. Differential migration and an endocrine response to stress in wintering dark-eyed juncos (*Junco hyemalis*). Proc R Soc Lond B Biol Sci 267:1889–1896.

Holberton R.L., P.P. Marra, and F.R. Moore. 1998. Endocrine aspects of physiological condition, weather and habitat quality in landbird migrants during the non-breeding period. Proc Int Ornithol Congr 22:847–866.

Holberton R.L., J.D. Parrish, and J.C. Wingfield. 1996. Modulation of the adrenocortical stress response in Neotropical migrants during autumn migration. Auk 113:558–564.

Hood L.C., P.D. Boersma, and J. Wingfield. 1998. The adrenocortical response to stress in incubating Magellanic penguins (*Spheniscus magellanicus*). Auk 15:76–84.

Jenni-Eiermann S. and L. Jenni. 1991. Metabolic responses to flight and fasting in night-migrating passerines. J Comp Physiol B 161:465–474.

John T.M. 1965. A histochemical study of adrenal corticoids in the pre- and post-migratory wagtail, *Motacilla alba* and *M. flava*. Pavo 4:9–14.

Joseph M.M. and A.H. Meier. 1973. Daily rhythms of plasma corticosterone in the common pigeon, *Columba livia*. Gen Comp Endocrinol 20:326–330.

Jukema J., T. Piersma, J.B. Hulscher, E.J. Bunskoeke, A. Koolhaas, and A. Veenstra. 2001. Goudplevieren en wilsterflappers: eeuwenoude fascinatie voor trekvogels. Fryske Akademy, Ljouwert/Koninklijke Nederlandse Natuurhistorische Vereniging Uitgeverij, Utrecht (in press).

King B.M. 1987. Glucocorticoids and hypothalamic obesity. Neurosci Biobehav Rev 12:29–37.

Kitaysky A.S., J.F. Piatt, and J.C. Wingfield. 1999*a*. The adrenocortical stress-response of black-legged kittiwake chicks in relation to dietary restriction. J Comp Physiol B 169:303–310.

Kitaysky A.S., J.C. Wingfield, and J.F. Piatt. 1999*b*. Dynamics of food availability, body condition and physiological stress response in breeding black-legged kittiwakes. Funct Ecol 13: 577–584.

Koopman K. and J.B. Hulscher. 1979. Catching waders with a "wilsternet." Wader Study Group Bull 26:10–12.

Landys M.M., T. Piersma, G.H. Visser, J. Jukema, and A. Wijker. 2000. Water balance during real and simulated long-distance migratory flight in the bar-tailed godwit. Condor 102: 645–652.

Lauber J.K., J. Vriend, and T. Oishi. 1987. Plasma corticosterone in chicks reared under several lighting schedules. Comp Biochem Physiol A 86:73–78.

Lorenzen L.C. and D.S. Farner. 1964. An annual cycle in the adrenal gland of the white-crowned sparrow, *Zonotrichia leukophrys gambelii*. Gen Comp Endocrinol 4:253–263.

Meier A.H. and D.D. Martin. 1971. Temporal synergism of corticosterone and prolactin controlling fat storage in the white-throated sparrow, *Zonotrichia albicollis*. Gen Comp Endocrinol 17:311–318.

Mizrahi D.S., R.L. Holberton, and S.A. Gathreaux, Jr. 2001. Patterns of corticosterone secretion in migrating semipalmated sandpipers at a major spring stopover site. Auk 118: 79–91.

Naik D.V. and J.C. George. 1963. Histochemical demonstrations of increased corticoid level in the adrenal of *Sturnus roseus* (Linneus) towards the migratory phase. Pavo 1: 103–105.

Nazir M.I., H.A. Rizvi, and S.S. Ali. 1988. Effect of corticone on the lipid composition of adipose tissue, plasma and liver in a lizard. Pak J Sci Ind Res 31:706–710.

O'Reilly K.M. 1995. Ecological Basis of Endocrine Phenomena: Field Studies of *Scolopacidae* as Model Systems. PhD diss. University of Washington.

Packard G.C. and T.J. Boardman. 1987. The misuse of ratios to scale physiological data that vary allometrically with body size. Pp. 216–239 in M.E. Feder, A.F. Bennett, W.W. Burggren, and R.B. Huey, eds. New Directions in Ecological Physiology. Cambridge University Press, Cambridge.

———. 1988. The misuse of ratios, indices, and percentages in ecophysiological research. Physiol Zool 61:1–9.

Peczely P. 1976. Etude circanuelle de la fonction corticosurrenalienne chez les especes de passereaux migrants et non-migrants. Gen Comp Endocrinol 30:1–11.

Piersma T. 1987. Hink, stap of sprong? Reisbeperkingen van arktische steltlopers door voedselzoeken, vetopbouw en vliegsnelheid. Limosa 60:185–191.

———. 1998. Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fueling and flight? J Avian Biol 29:511–520.

Piersma T., J.M. Everaarts, and J. Jukema. 1996. Build-up of red blood cells in refueling bar-tailed godwits in relation to individual migratory quality. Condor 98:363–370.

Piersma T. and J. Jukema. 1990. Budgeting the flight of a long-distance migrant: changes in nutrient reserve levels of bar-tailed godwits at successive spring staging sites. Ardea 78: 315–337.

———. 1993. Red breasts as honest signals of migratory quality in a long-distance migrant, the bar-tailed godwit. Condor 95:163–177.

Piersma T., M. Klaassen, J.H. Bruggemann, A.-M. Blomert, A. Gueye, Y. Ntiamoa-Baidu, and N.E. van Brederode. 1990. Seasonal timing of the spring departure of waders from the Banc d'Arguin, Mauritania. Ardea 78:123–134.

Piersma T., A. Koolhaas, and A. Dekinga. 1993. Interactions between stomach structure and diet choice in shorebirds. Auk 110:552–562.

Piersma T. and M. Ramenofsky. 1998. Long-term decreases of corticosterone in captive migrant shorebirds that maintain seasonal mass and moult cycles. J Avian Biol 29:97–104.

Piersma T., J. Reneerkens, and M. Ramenofsky. 2000. Baseline corticosterone peaks in shorebirds with maximal energy stores for migration: a general preparatory mechanism for rapid behavioral and metabolic transitions? Gen Comp Endocrinol 120:118–126.

Piersma T. and S. van de Sant. 1992. Pattern and predictability of potential wind assistance for waders and geese migrating from West Africa and the Wadden Sea to Siberia. Ornis Svecica 2:55–66.

Prokosch P. 1988. Das Schleswig-Holsteinische Wattenmeer als Frühjahrs-Aufenthaltsgebiet arkitscher Watvogel-Populationen am Beispiel von Kiebitzregenpfeifer (*Pluvialis squatarola*, L. 1758), Knutt (*Calidris canutus*, L. 1758) und Pfuhlschnepfe (*Limosa lapponica*, L. 1758). Corax 12:274–442.

Ramenofsky M. 1990. Fat storage and fat metabolism in relation to migration. Pp. 214–231 in E. Gwinner, ed. Bird Migration: Physiology and Ecophysiology. Springer, Berlin.

Ramenofsky M., T. Piersma, and J. Jukema. 1996. Plasma corticosterone in bar-tailed godwits at a major stop-over site during spring migration. Condor 97:580–585.

Romero L.M., M. Ramenofsky, and J.C. Wingfield. 1997. Season and migration alters the corticosterone response to capture and handling in an arctic migrant, the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). Comp Biochem Physiol C 116:171–177.

Santana P., S.F. Akana, E.S. Hanson, A.M. Strack, R.J. Sebastian, and M.F. Dallman. 1995. Aldosterone and dexamethasone both stimulate energy acquisition whereas only the glucocorticoid alters energy storage. Endocrinology 136:2214–2222.

Schwabl H., F. Bairlein, and E. Gwinner. 1991. Basal and stress-induced corticosterone levels of garden warblers, *Sylvia borin*, during migration. J Comp Physiol B 161:576–580.

SPSS. 1997. SigmaStat. Version 2.0. SPSS, Chicago.

Sokal R.R. and F.J. Rohlf. 1995. Biometry: The Principles and Practice of Statistics in Biological Research. 3d ed. W. H. Freeman, New York.

Tempel D.L. and S.F. Leibowitz. 1989. PVN steroid implants: effect on feeding patterns and macronutrient selection. Brain Res Bull 23:553–560.

Tsipoura N., C.G. Scanes, and J. Burger. 1999. Corticosterone and growth hormone levels in shorebirds during spring and fall migration stopover. J Exp Zool 284:645–651.

Veldhuis H.D., R. de Kloet, I. van Zoest, and B. Bohus. 1982. Adrenalectomy reduces exploratory activity in the rat: a specific role of corticosterone. Horm Behav 16:191–198.

Wilkinson L. 2000. SYSTAT for Windows: Statistics. Version 10. SYSTAT, Evanston, Ill.

Wingfield J.C., P. Deviche, S. Sharbaugh, L.B. Astheimer, R. Holberton, R. Suydam, and K. Hunt. 1994. Seasonal changes of the adrenocortical responses to stress in redpolls, *Acanthis flammea*, in Alaska. J Exp Zool 270:372–380.

Wingfield J.C., D.L. Maney, C.W. Breuner, J.D. Jacobs, S. Lynn, M. Ramenofsky, and R.D. Richardson. 1998. Ecological bases of hormone-behavior interactions: the "emergency life history stage." Am Zool 38:191–206.

Wingfield J.C. and R. Ramenofsky. 1999. Hormones and the behavioral ecology of stress. Pp. 1–51 in P.H.M. Balm, ed. Stress Physiology in Animals. CRC Press, Boca Raton, Fla.

Wingfield J.C., H. Schwabl, and P.W. Mattocks, Jr. 1990. Endocrine mechanisms of migration. Pp. 232–256 in E. Gwinner, ed. Bird Migration: Physiology and Ecophysiology. Springer, Berlin.

Wingfield J.C., C.M. Vleck, and M.C. Moore. 1992. Seasonal changes in the adrenocortical response to stress in birds of the Sonoran Desert. J Exp Zool 264:419–428.

Zar J.H. 1996. Biostatistical Analysis. 3d ed. Prentice Hall, Upper Saddle River, N.J.